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| 10/744,007 | 12/24/2003 | Tetsuo Nagano | P24350 | 9418 |
| 7055 | 7590 | 12/04/2006 | EXAMINER | |
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1743

DATE MAILED: 12/04/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary

Application No.

10/744,007

Applicant(s)

NAGANO ET AL.

Examiner

Arlen Soderquist

Art Unit

1743

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 21 September 2006.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 9-14 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 9-14 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
 - ☐ Certified copies of the priority documents have been received in Application No. _____.
 - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

Art Unit: 1743

1. Claim 14 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In claim 14 a method of measuring nitric oxide is found in the preamble however the body of the claim does not contain any measurement steps to allow the method to actually measure the nitric oxide. Thus the claim is incomplete and misses at least one required step.

2. Applicant is advised that should claim 9 be found allowable, claim 11 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). Both claim are simply claiming the compound of claim 9 since there is nothing in addition to the compound of claim 9 that is required for the reagent of claim 11.

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

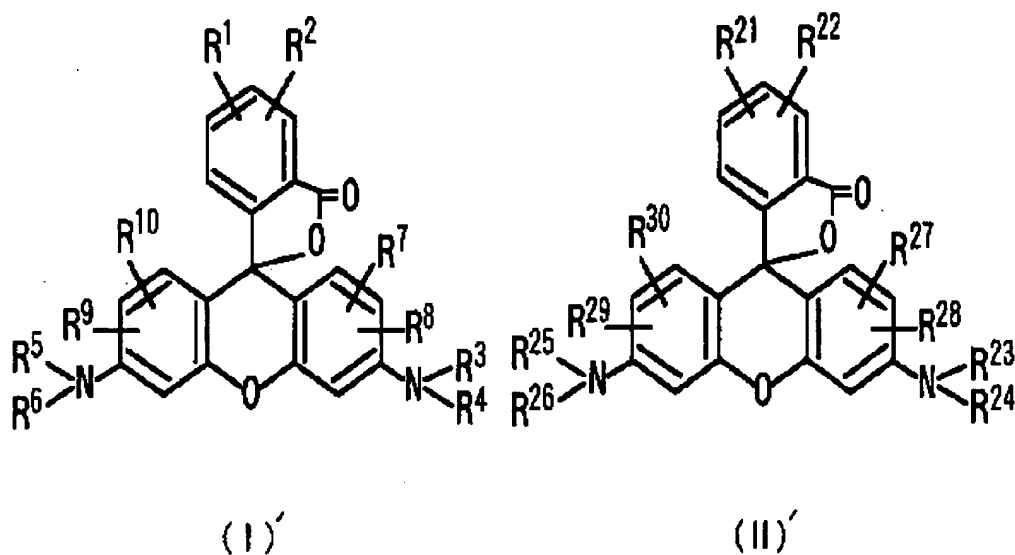
The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

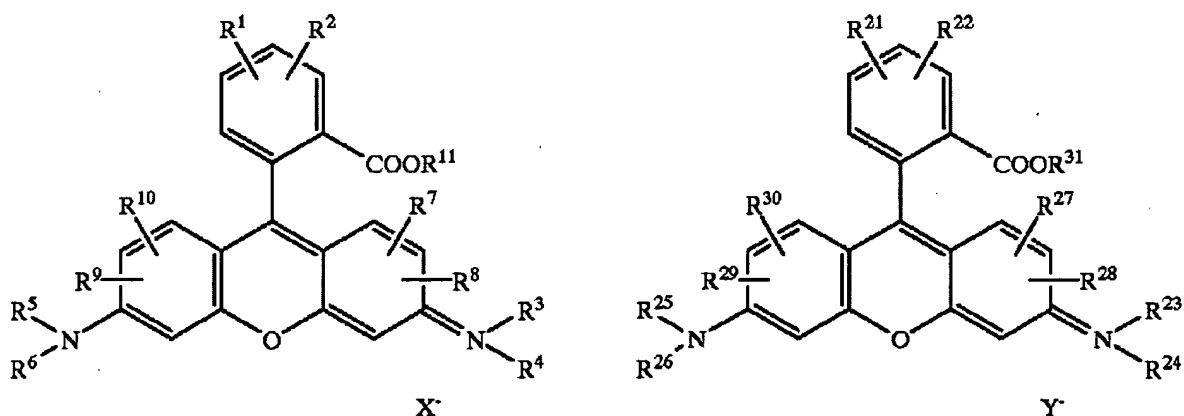
4. Claims 9-14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nagano (CA 2,295,880 newly cited and applied) in view of Kojima (Angewandte Chemie, International Edition 1999, newly applied and hereinafter referred to as Kojima '99), Kojima (Biological & Pharmaceutical Bulletin 1997, newly cited and applied and hereinafter referred to as Kojima '97) and Chen (newly cited and applied). In the publication Nagano teaches diaminorhodamine derivatives that are reactive with nitrogen monoxide to form a fluorescent compound. Relevant

Art Unit: 1743

to the instant claims are lactone ring formulas (I)' and (II)' found on page 13 and reproduced below.



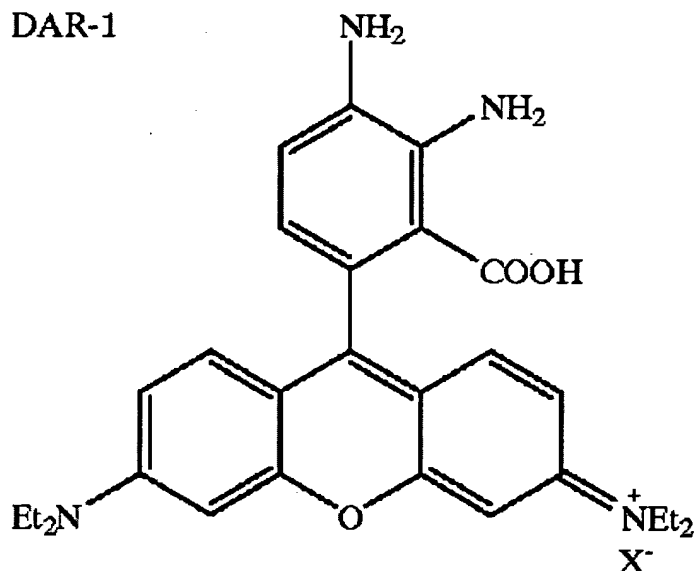
Page 12 in the last paragraph teach that these lactone ring structures correspond to formulas (I) and (II) including the meanings of R-groups R¹ to R¹⁰ and R²¹ to R³⁰. Formulas (I) and (II) are found on pages 5 and 6 respectively and are reproduced below in a format that corresponds to formulas (I)' and (II)' above.



Beginning on page 7, the best mode is described for formulas (I) and (II). In formula (I), R¹ and R² represent amino groups at adjacent positions on the phenyl ring. R¹ and R² are preferably

Art Unit: 1743

non-substituted amino groups, or one of R^1 and R^2 may be a monosubstituted amino group (this covers the instantly claimed compounds of claims 9-11). Examples of the substituent present on the amino group include, for example, a linear or branched C_{1-18} alkyl group (preferably, a C_{1-6} alkyl group). The C_{1-6} alkyl may be linear or branched unless otherwise specifically indicated. Specific examples thereof include, for example, methyl group (covers the structure of claims 9-10), ethyl group, n-propyl group, isopropyl group, n-butyl group, sec-butyl group, tert-butyl group. R^3 , R^4 , R^5 and R^6 each independently represent a C_{1-6} alkyl group, and alkyl groups for these groups may be the same or different. For example, compounds wherein R^3 , R^4 , R^5 and R^6 are ethyl groups in a preferred embodiment. R^7 , R^8 , R^9 and R^{10} each independently represent hydrogen atom, a C_{1-6} alkyl group, an allyl group ($CH_2=CH-CH_2-$), or a halogen atom with each of R^7 , R^8 , R^9 and R^{10} preferably being a hydrogen atom. In formula (II), R^{21} and R^{22} may bind to each other to represent a group $-N=N-NR^{41}-$ that forms a ring at adjacent positions on the phenyl ring. R^{41} represents hydrogen atom, a linear or branched C_{1-18} alkyl group (preferably, a C_{1-6} alkyl group covering the structure of claims 12-13), or a C_{1-6} alkyl group substituted with an optionally-substituted aryl group. R^{23} , R^{24} , R^{25} and R^{26} each independently represent a C_{1-6} alkyl group, and the alkyl groups represented by these groups may be the same or different. For example, compounds wherein R^{23} , R^{24} , R^{25} and R^{26} are ethyl groups in preferred embodiments. R^{27} , R^{28} , R^{29} and R^{30} each independently represent hydrogen atom, a C_{1-6} alkyl group, an allyl group, or a halogen atom with each of R^{27} , R^{28} , R^{29} and R^{30} preferably being a hydrogen atom. Among the compounds represented by the formula (II), those wherein R^{21} and R^{22} bind to each other to form a group represented by $-N=N-NR^{41}-$ that forms a ring at adjacent positions on the phenyl ring can be produced by allowing a compound of the aforementioned formula (I) to react with nitric oxide. See the paragraph bridging pages 4-5 for an explanation that the ring formed through this reaction with nitric oxide is a triazole ring. Those compounds have strong fluorescence and are useful for the measurement of nitric oxide. See the paragraph bridging pages 13-14 for a description of the method. The instantly claimed compounds are completely covered by appropriate selection of R^1 to R^{10} and R^{21} to R^{30} in formulas (I)' and (II)'. Pages 10-11 show the synthesis of three compounds of formula (I). Of particular relevance to the instant claims is the compound labeled DAR-1 reproduced below. This compound has the preferred



forms of R¹ to R¹⁰.

ring structure of formula (I)' based on this structure differs from instant claim 9 in having a methyl group substitution on the amino group at the top position. A lactone ring structure of formula (II)' based on this structure differs from instant claim 12 in having a methyl group substitution for R⁴¹ when R²¹ and R²² represent the -N=N-NR⁴¹- forming a ring at adjacent positions on the phenyl ring.

In the paper Kojima '99 teaches fluorescent indicators for imaging nitric oxide production. They developed diaminofluoresceins (DAFs), such as DAF-2, as fluorescent indicators for NO. Under aerobic conditions, DAFs can trap NO to yield highly fluorescent triazolo fluoresceins (DAF-T5) by nitrosation and dehydration. This mechanism is convenient because it does not interfere with signal transduction. Under physiological conditions, DAF-Ts are not formed in the absence of NO, which is why DAFs can be used for direct detection of NO produced by cells. However, during the application of DAF-2 for NO imaging in vascular endothelial cells, they encountered the problem of pH dependency of the fluorescence intensity of DAF-Ts, which makes it difficult to monitor small changes in intracellular NO levels after a stimulus that shifts the intracellular pH level. They synthesized fluorinated fluorescein derivatives that are more resistant to photobleaching than fluorescein and can be efficiently excited with the 488-nm spectral line of the argon-ion laser used in confocal laser scanning

Art Unit: 1743

microscopes. The paragraph bridging the columns of page 3210 teaches the introduction of a methyl group (see scheme 2) to correct a fluorescence instability around neutral pH that may arise from the triazole proton (-N=N-NH-).

In the paper Kojima '97 discusses the development of a fluorescent indicator for the bioimaging of nitric oxide. The N-nitrosation of newly designed and synthesized 4-((3-amino-2-naphthyl)aminomethyl)benzoic acid (DAN-1) by NO yielded the highly fluorescent triazole-form. This compound was made to prevent the known diaminonaphthalene from leaking through the cell membranes (see paragraph bridging the columns of page 1229). The membrane permeable ester derivative of DAN-1 (DAN-1 EE) was applied to the imaging of NO produced in activated rat aortic smooth muscle cells. After DAN-1 EE has been loaded into cells, the ester bond is hydrolyzed by intracellular esterase, yielding original DAN-1 with less permeability. The fluorescence intensity of the cells loaded with DAN-1 EE increased according to NO production (see figure 3). The paragraph bridging the columns of page 1231 teaches that the lack of a proton on the triazole of the developed fluorescent compound resulted in no change of fluorescent intensity around neutral pH compared to the increased fluorescence at alkaline pH due to the deprotonation of the triazole in the known diaminonaphthalene reagent.

In the paper Chen studied the fluorescent species of 7-azaindole and 7-azatryptophan in water. A study of the fluorescence lifetimes and quantum yields of 7-azaindole and its methylated derivatives N1-methyl-7-azaindole (1M7AI, C in figure 1) and 7-methyl-7H-pyrrolo[2,3-b]pyridine (7M7AI, D in figure 1) in water was performed in order to explain the observation that the fluorescence spectrum of 7-azaindole apparently consists of one band ($\lambda_{\text{max}} = 386 \text{ nm}$) whereas in alcohols the spectrum is bimodal (e.g., for methanol, $\lambda_{\text{max}} = 374, 505 \text{ nm}$). Careful measurements of the fluorescence decay as a function of emission wavelength indicate a small amplitude of an ~ 70 -ps decaying component at the bluer wavelengths and a rising component of the same duration at the redder wavelengths. The small amplitude component, which comprises no more than 20% of the fluorescence decay, is attributed to excited-state tautomerization that is mediated by the solvent. Particular attention is paid to the pH dependence of the fluorescence lifetimes and yields. Upon tautomerization, the basic 1-nitrogen (N1) of 7-azaindole is rapidly protonated giving rise to a species whose emission maximum is at $\sim 440 \text{ nm}$. The fluorescence emission maximum and lifetime of 7-azaindole is dominated by the 80% of the

Art Unit: 1743

solute molecules that are blocked by unfavorable solvation from executing excited-state tautomerization. 1M7AI and 7M7AI represent the two tautomers and are locked into the configurations by the methyl substitutions at the respective nitrogens. Figure 10 shows the pH dependency of the fluorescence for the 7-azaindole molecule and figure 11 shows the dependency for the 1M7AI and 7M7AI molecules. It is clear that substitution of the proton with a methyl group removes some dependency of the fluorescence on the pH.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a methyl group for R⁴¹ in the structure of the Nagano lactones because as taught by Kojima '99 the substitution eliminates fluorescence instability that is known to occur when a proton is present on the triazole ring. It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a methyl group for R⁴¹ in the structure of the Nagano lactones because as taught by Kojima '97 the substitution eliminates fluorescence instability that is known to occur when a proton is present on the triazole ring and the substitution would be recognized as not required to prevent the molecule from leaking through the cell membrane. It would have been obvious to one of ordinary skill in the art at the time the invention was made to use a methyl group for R⁴¹ in the structure of the Nagano lactones because as taught by Chen the substitution eliminates fluorescence instability by locking the structure in one tautomer when a proton is present on a nitrogen containing ring capable of tautomerization.

5. The terminal disclaimer filed on September 21, 2006 disclaiming the terminal portion of any patent granted on this application which would extend beyond the expiration date of US patent 6,756,231 has been reviewed and is accepted. The terminal disclaimer has been recorded.

6. Applicant's arguments with respect to claims 9-14 have been considered but are moot in view of the new ground(s) of rejection.

7. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure. The additionally cited art relates to tautomerism of 1,2,3 triazoles and fluorescent indicators.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Arlen Soderquist whose telephone number is (571) 272-1265. The examiner can normally be reached on Monday-Thursday and Alternate Fridays.

Art Unit: 1743

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Jill Warden can be reached on (571) 272-1267. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.



Arlen Soderquist
Primary Examiner
Art Unit 1743